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Organic Acids Toxicity on Seedling Attributes of Anoxia Tolerant Rice Genotypes Grown in Hypoxia

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Abstract: Sprouted seeds of anoxia-tolerant rice genotypes (IR41996-50-2-1-3, IR50363-61-1-2-2, BR736-20-3-1, RP1669-1529-4254) were sown in the para-film sealed test tubes containing different acid solutions and were allowed to grow for 7 days at 30°C at pH5 and 7 in the dark. The variation in organic acid concentration for 50% growth inhibition (C_{50}) of seedling attributes was evident. First leaf survival (%), and the growth of the other seedling organs could be improved in pH 7 for some genotypes. RP1669-1529-4254 showed a higher C_{50} value in acetic acid for pH 5 and in propionic acid and butyric acid for both pH 5 and pH 7. It appears that this genotype has more tolerance and wider adaptability than the others under oxygen depleted conditions. First leaf survival (%) was more tolerant as compared to first leaf length, plant height and root length. Among VFAs, propionic acid was the most toxic, followed by butyric and acetic acid.

Key words: Rice, seedling growth, toxicity, VFA,

Introduction

An anoxia-tolerant rice genotype is expected to establish seedling under poorly aerated lowland conditions (Biswas and Yamauchi, 1997; Yamauchi and Biswas, 1997). Despite using these varieties, variation of seedling establishment from one location to the other is still evident (Yamauchi and Biswas, 1996; Yamauchi *et al.*, 2000). This phenomenon could be attributed to accumulated volatile fatty acids (VFA) (Rao and Mekkelesen, 1977). Among the VFAs, lower carbon aliphatic monobasic acids like formic, acetic, propionic, butyric and lactic acids were observed to cause injury to rice plants (Takijima, 1964). Considerable studies were done on the effect of these organic acids on the growth of rice. Currently, little information is available on the level of organic acid on the process of seedling development under hypoxic conditions of the anoxia tolerant rice genotype seedling. Previously, Biswas *et al*. (2001) reported on the effects of some VFA and of pH on a few anoxia tolerant rice genotypes. While this study generated some information, the present study was conducted to obtain very specific information relating the toxic level of organic acids regarding different seedling organs of the anoxia-tolerant rice genotypes to develop an appropriate technology for direct seeded lowland rice.

Materials and Methods

The studies were conducted in the laboratory of Crop Science, Faculty of Agriculture, Yamagata University, Japan in 2000.

Plant materials: Four indica type anoxia-tolerant genotypes (IR41996-50-2-1-3, IR50363-61-2-2, RP1669-1529-4254, and BR736-20-3-1) (Yamauchi *et al.*, 1993, and Yamauchi *et al.*, 2000) were obtained from the International Rice Research Institute.

Germination percentage and germination rate (Krishnasamy and Seshu, 1989) at 30°C were respectively, IR41996-50-2-1-3, 95.0%, 0.99, IR50363-61-1-2-2, 100.0%, 1.0, RP1669-1529-4254, 99.0%, 0.99; BR736-20-3-1, 100%, 1.00: and Haenuki, 99.00%, 0.99.

Organic acids: For simplicity, acetic, propionic and butyric acids from the VFA group were considered for this study. Acid concentrations were decided on a trial and error basis. The concentrations used for acetic acid were 0, 4, 8 and 12 mM. The concentrations for propionic acid were 0,1, 2, and 3mM and for butyric acid were 0, 2, 4, and 6 mM.

NaOH and HCl were used to adjust the pH (7 or 5) of solutions under consideration. Five sprouted seeds (surface sterilized in 2% NaOCl for 15 minutes), were sown in the test tubes (100X25-mm). Each test tube contained 9ml of specified organic acid solution, equivalent to a 25 mm solution column above the seeds, to maintain hypoxic conditions. The test tubes were then sealed with para film, wrapped in aluminum foil, and kept in an incubator at 30°C. The seedlings were allowed to grow for 7 days. The solutions were replaced with fresh ones every 2 days. The experiments were run in a three replicated completely randomized design.

First leaf survival (%), first leaf length, plant height and root length were observed. The concentrations required for 50% (designated as C_{50} in this article) growth inhibition of the seedling attributes (may be 1st leaf survival (%), or leaf/root length, or plant height) were used to estimate the toxicity level of organic acids. We borrowed this concept from a widely used term in biological research, LD_{50} (lethal dose 50%), defined as the dose that kills 50% of the animals in an experiment. Takijima (1960) used this concept to study the nature of root growth inhibition due to organic acids. The quadratic relations between seedling attributes and organic acids were used to estimate the C_{50}s as follows:

$$Y = ax^2 \pm bx \pm c \ldots (I)$$

where Y = 50% of first leaf survival or first leaf length or plant height or root length as compared with the control treatment, x = C_{50}, a and b are the rate of curvilinear and linear coefficients respectively, while c is the intercept.

The solution for x is given as follows:

$$x = \{-b \pm \sqrt{b^2 - 4a\,(c-Y)}\}/2a \ldots (ii)$$

We found this equation convenient to estimate "x" values as the coefficients used for C_{50} evaluation and coefficients of determination (R^2) were highly significant (Fig. 1).

It may be mentioned that equations presented in the figures were based on the mean values over the replications. However, C_{50}s were estimated replication wise so that analysis of variance could be performed.

Results and Discussion

First leaf survival (%), first leaf length, plant height and root length decreased polynomially with the increase in acid

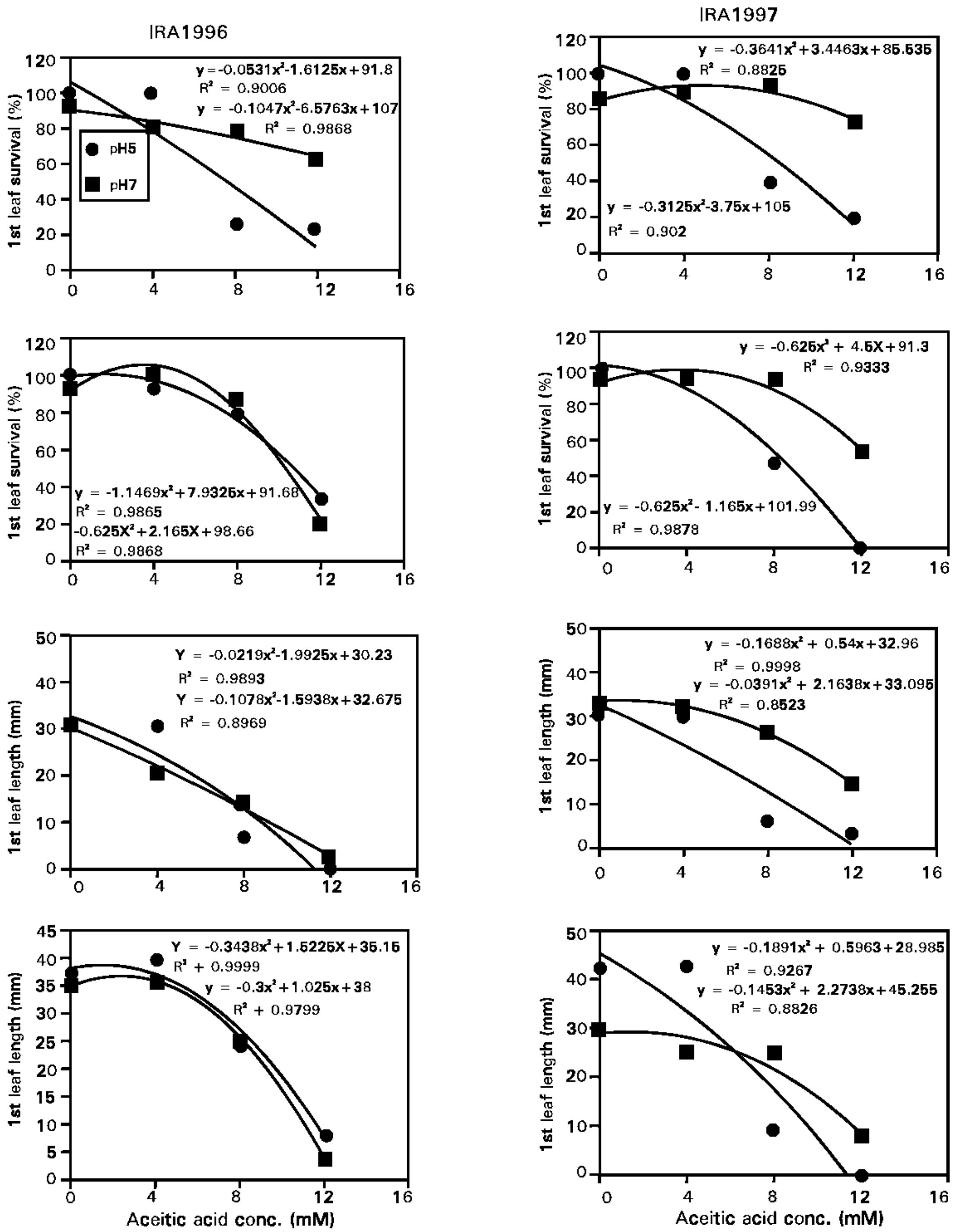

Fig. 1: Dependence of first leaf survival and first leaf length of different rice genotypes to acetic acid and pH.

Table 1: C_{50} of different genotypes as affected by VFA, seedling attributes and pH

Acid	Genotype (G)	1st leaf survival (%)		1st leaf length (mm)		Plant height (mm)		Root length (mm)	
		pH (P)		pH (P)		pH (P)		pH (P)	
		5	7	5	7	5	7	5	7
Acetic	IR41996	7.71	17.49	6.93	7.28	4.67	6.08	7.48	8.10
	IR50363	8.56	16.49	7.21	11.60	7.14	9.75	7.92	10.64
	RP1669	10.75	10.61	9.92	9.70	7.72	7.79	7.98	9.69
	BR736	8.52	12.49	7.24	10.31	5.97	9.79	5.97	9.25
$LSD_{0.05}$ (GXP)		0.52		0.84		0.55		0.66	
Propionic	IR41996	0.75	1.02	0.62	0.65	0.59	0.59	1.04	0.66
	IR50363	0.65	3.25	1.06	1.89	1.24	2.58	2.35	3.77
	RP1669	2.21	3.91	2.81	3.05	2.04	3.04	2.01	3.42
	BR736	0.75	0.76	1.90	0.69	0.66	0.62	1.14	0.89
$LSD_{0.05}$ (GXP)		0.55		1.00		0.57		0.57	
Butyric	IR41996	4.61	9.76	3.43	6.00	2.81	2.95	1.54	1.70
	IR50363	2.80	6.01	2.25	5.04	1.91	2.86	1.53	1.99
	RP1669	5.21	7.02	4.02	5.06	3.13	4.07	1.59	2.87
	BR736	4.65	7.33	3.59	5.01	2.66	3.02	1.26	1.36
$LSD_{0.05}$ (GXP)		0.75		0.72		0.62		0.67	

concentrations. The trend of first leaf survival (%) and first leaf length in IR41996-50-2-1-3, IR50363-61-1-2-2, RP1669-1529-4254 and BR736-20-3-1, affected by acetic acid, is presented in Fig. 1. pH7 showed significantly higher C_{50} values of first leaf survival (%) at the higher acetic acid concentrations for IR41996-50-2-1-3, IR50363-61-1-2-2 and BR736-20-3-1 as compared with pH5 (Table 1). Like first leaf survival percentage, all of the seedling attributes were significantly related to acetic, propionic and butyric acids (Figures are not shown).

Compared with other genotypes, IR50363-61-1-2-2 and RP1669-1529-4254 exhibited significantly higher C_{50} at pH7 in propionic acid. With little exception, pH7 also executed significantly higher C_{50} in butyric acid for all seedling attributes. In acetic acid, RP1669-1529-4254 and IR50363-61-1-2-2, had higher C_{50} for their attributes in pH5 and pH7 respectively. RP1669-1529-4254 showed the highest C_{50} at both pH levels in respect to most of the seedling attributes in propionic and butyric acid. In acetic and butyric acid, C_{50} for first leaf survival (%) was higher than the other attributes (Table 1).

The concentration of organic acids increases with flooding and reaches a peak within several weeks, and then decreases to insignificant levels (Ponnamperuma, 1976). The kinetics of these acids varies with the physical-chemical properties of the soil. Incorporation of organic matter such as green manure, glucose and straw, promotes the production of organic acids in submerged soils (Gotoh and Onikura, 1971).

Cho and Ponnamperuma (1971) reported that the concentration of organic acid reached a maximum of 10 to 36mM within 1 to 3 weeks after incubation. Among the organic acids, acetic acid is predominant, followed by propionic acid and butyric acid (Watanabe, 1984). Yamane and Sato (1970) found that acetic acid and butyric acid accumulated to levels of 13.5 and 5.5mM/kg soil respectively, when Italian rye grass (10.6t/ha) was incorporated into the rice field. Watanabe (1984) reported that the maximum accumulation of acetic, propionic and butyric acids could be 43.8, 1.3 and 4.8mM/kg soil respectively. The C_{50s} obtained in this study for acetic acid were quite low as compared with reported values. C_{50} in propionic and butyric acid for all seedling attributes were spread around their reported values. So, there is a possibility of affecting seedling growth by VFA under lowland conditions.

We observed higher C_{50} for first leaf survival (%) as compared to other seedling organs. That means, despite the first leaf survival, the mere presence of any of these acids might affect subsequent stages of seedling growth.

The higher pH helped better seedling attributes for some genotypes by enhancing tolerance level. RP1669-1529-4254 hardly responded to pH7 in the predominantly occurring acetic acid. However, the genotype maintained significantly higher C_{50} value in acetic acid for pH5 and in propionic for both pH 5 and pH7. This acid, though not significantly, showed higher C_{50} value at pH 5 and 7 in butyric acid. A genotype showing higher C_{50} had better tolerance to organic acids than the others. Therefore, it may be stated that RP1669-1529-4254 has more tolerance and wider adaptability to these conditions. For other genotypes, tolerance to volatile fatty acids vary with pH.

Variation among the anoxia tolerant genotypes in regard to 50% growth inhibition due to organic acids is quite evident from the study. First leaf survival (%), and other subsequent seedling organs could be improved in pH 7 for some genotypes. RP1669-1529-4254 showed higher C_{50} with acetic acid for pH 5 and in propionic and butyric acid for both pH 5 and 7. It appears that this genotype has more tolerance and wider adaptability to lowland conditions.

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